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EFFECTS OF RADIATION ON THE RESPIRATORY METABOLISM OF WHITE BLOOD CELLS

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Lieutenant, U. S. Coast Guard
M.Sc., The Ohio State University, 1951.

THESIS
P29

EFFECTS OF RADIATION ON THE RESPIRATORY METABOLISM OF WHITE BLOOD CELLS

Abstract of

A Thesis

Presented in Partial Fulfillment of the Requirements

for the degree Master of Science

by

PAUL REED PEAK JR., B.S.

The Ohio State University

1951

Thesis
P29

THE EFFECTS OF RADIATION ON THE RESPIRATORY METABOLISM OF WHITE BLOOD CELLS

PAUL REED PEAK JR.

B.S., The United States Coast Guard Academy, 1944

Department of Physics

(Approved by William G. Myers, M.D.)

Heterophilic polymorphonuclear granulocytes from the peritoneal exudate and lymphocytes from the appendix of the rabbit were irradiated with x- and beta-radiation *in vitro*, and the subsequent oxygen uptake of the cells was taken as a quantitative measure of the radiation effects. Mean respiration values of the non-irradiated controls expressed in microliters of oxygen per million cells per hour were found to be two hundred sixty-two one thousandths for the granulocytes and six hundred sixty-two one thousandths for the lymphocytes.

The dose of unfiltered x-radiation that reduced granulocyte oxygen consumption to fifty percent of control was twenty-three thousand roentgens, and the respiration curve followed an exponential depression after an initial stimulation. The lymphocytes are much more radiosensitive; their fifty percent dose was six thousand roentgens, and the respiration curve was exponential after an initial depression.

Using granulocyte suspensions as a biological indicator, this study found the relative biological effectiveness

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of beta radiation to be about one-half as great as x-radiation.

Radiation effects on respiration were shown to be strongly dependent upon the pH of the suspending medium, and directly proportional to the hydrogen ion concentration over a range of one pH unit around the pH of blood.

The radiation effects varied inversely with granulocyte concentration in the suspension, over the range from one to sixteen times their concentration in peripheral blood.

Irradiation at ten to twenty degrees centigrade had no definite effect on respiration, nor did the "incubation time" after irradiation. A reduced radiation effect was obtained when the cells were irradiated with filtered x-radiation.

It is concluded that destruction of the peripheral white blood cells is a minor factor in radiation injury, even though lymphoid and myeloid are the two most radiosensitive tissues.

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Introduction

Changes in the blood were among the first effects of exposure of the body to radiation which were recognized after Roentgen's original discovery. The best present index of radiation damage is the change in blood and blood-forming organs. Nevertheless, the mechanism of radiation injury to the blood cells is still not fully understood. Nor is the relative effectiveness of irradiation by the different ionizing particles finally established. Some of the effects of irradiating peripheral white blood cells _in vitro_ have been investigated. Granulocyte and lymphocyte suspensions obtained from rabbits were irradiated either with x- or beta-radiation. The subsequent oxygen uptake of the cells was used as a quantitative measure of the radiation effects on the respiration.

Historical Review

A. _In Vivo_ Irradiation

The typical result of massive whole-body irradiation on the granulocyte series is an initial moderate leukocytosis followed by a drop in the cell count. Hagen, Jacobson, Murray, and Lear (5) found two peaks, at eight and at twenty-four hours, in the heterophile count of rabbits receiving 500 roentgens of x-radiation. These rises were followed by a granulocytopenia which disappeared

in eight to ten days. The transient leukocytosis is probably due to a mobilization of the granulocytes throughout the body. The subsequent leukopenia is attributed to the radiation damage to the hematopoietic tissue in the bone marrow.

Glenn (2, 3) used the phagocytic index as a measure of the effect of radiation on rabbits. He found an increase in the index 48 to 96 hours following 100 roentgens of x-radiation delivered to the thigh.

The lymphocytes are the most sensitive of the peripheral blood cells. After whole-body exposure to single doses of radiation a lymphopenia appears within twenty-four hours. Stearner, Simmons, and Jacobson (20) found a significant drop in the lymphocyte count of the rat after exposure to 25 roentgens. Recovery following a non-lethal dose began, depending on the dose, within a few hours and was complete in a few weeks.

Hagen, et al, also observed by fixed sections the ring of lymphatic tissue in the rabbit appendix after in vivo exposure to 400 roentgens. Degeneration of the small, and to a lesser extent, the medium and the large lymphocytes began at one hour after irradiation, increased to a maximum at eight hours, and ended at twenty-four hours. The resulting cellular debris was rapidly phagocytized by macrophages, while the heteroplastic regeneration of lymphocytes began within one hour.

De Bruyn (1) by means of histological studies after whole-body exposure, found the minimum detectable injury to rabbit lymph nodes after a dose of 50 roentgens.

B. *In Vitro* Irradiation

Studies on the radiation effects on white blood cells *in vitro* have been carried out by several authors. Jolly and Lacassagne (6) compared rabbit blood after *in vitro* and *in vivo* irradiation, and found no effect on count or motility of either the polymorphonuclear granulocytes or the lymphocytes in the *in vitro* samples. Spurling and Lawrence (19) used radium to deliver twice the erythema dose to citrated human blood. It was then examined for histological changes at fifteen minute intervals for one and one-half hours, without detecting any alteration in the leukocytes.

Osgood and Bracher (11) irradiated human bone marrow cultures containing lymphocytes as well as mature and immature cells of the granulocyte series. Their criterion was the total and differential count, expressed in percentage of the non-irradiated control, and they examined the cultures at daily intervals for a week following the exposure. At 400 roentgens, the lymphocyte count dropped immediately, reaching a minimum in three to four days, while the granulocyte count drop began at forty-eight hours, reaching a minimum in seven days. These

investigators found no significant variation in effect with variation in quality (HVL varied from 1.54 mm Cu to 0.36 mm Cu) or dosage rate (from 74 r/min to 7 r/min).

A refinement of the appearance criterion was used by Schrek (16) by adding eosin in Tyrode's solution to cell suspensions after irradiation, then counting the unstained cells, which were presumed viable. The suspensions were derived from rabbit bone marrow, spleen, and thymus, and from both lymphocytic and myelogenous leukemic human blood. Schrek found here that exposure to 1,000 roentgens caused lymphocyte death after a three hour latent period, but left the granulocytes unaffected.

Radiation effects on granulocytes specifically were looked for by Knott and Watt (7) using first the appearance in stained films and then the phagocytic power as an index. They found a radiation quality variation when oxalated human blood, held in a paraffin block to minimize backscatter, showed no damage after an exposure of two hours to deep x-rays, but definite damage appeared after fifteen minutes when blotting paper saturated in a metallic salt solution was placed directly over the liquid sample. Complete loss of phagocytosis to staphylococci was found after an exposure lasting thirty-five minutes, which demonstrated that the phagocytic function is a more sensitive index of cell damage than is microscopic appearance.

Gunz (4) studied cultures made from patients with chronic myeloid leukemia by total and differential counts and by the mitotic count. He found a significant reduction in the number of immature cells present forty-eight hours after an exposure to 100 roentgens, or twenty-four hours after 1,000 or 10,000 roentgens. But the mature granular cells were equally numerous in control and irradiated samples.

Work on lymphatic tissue suspensions has been done by Stenstrom and King (21), who irradiated mesenteric lymph node cultures at dosages up to 15,000 roentgens, and observed migration and ameboid movement over a period of a month. They found an inhibition of migration following exposure. Schrek (17) correlated dark field microscope observations of irradiated lymphatic suspensions from the thymus with fixed and stained spreads. He observed small vacuoles in one or a few focal areas in the nucleus which developed slowly, although at a faster rate in exposed than in control cells. He concluded that x-rays accelerate lymphocyte degeneration developing from focal intranuclear areas of hydration.

In summary, these observation show that lymphocytes and immature granulocytes are more radiosensitive than mature polymorphonuclear leukocytes. Good cultures of normal peripheral blood cells and a sensitive, quantitative index of radiation damage to mature cells are the major

techniques to be improved for further investigation on the action and mechanism of ionizing radiation on white blood cells.

C. White Cell Cultures.

There are several techniques available for obtaining the peripheral leukocytes for *in vitro* studies. Human leukocytes may be separated from normal or leukemic whole blood by leukocyte flotation (Vallee, Hughes, and Gibson, 23), by erythrocyte lysis (Singer, Silberbach, and Schwartz, 18), or by erythrocyte sedimentation (Minor and Burnett, 9). Human bone marrow cultures (Osgood and Bracher, 11) and "buffy coat" isolation from leukemic patients (Schrek, 16 and Gunz, 4) are methods which have been used by investigators for studying irradiation effects *in vitro*. Cultures made by these techniques are not erythrocyte free, nor is there an isolation of the different forms. The number of mature cells is low in all except the method which depended on the separation from normal whole blood, in which case the use of the agents such as albumin and gramicidin probably affects the morphology and physiology of the cells.

In the present study rabbit granulocytes were obtained from the peritoneal exudate, a technique credited to H. J. Hamburger (Mudd et al, 10; Singer et al, 18). This method, if not repeated at too frequent

intervals (Ponder and MacLeod, 13) gives mature heterophilic granulocytes (Class I or older) in relatively large quantity, with negligible admixture of other cell types.

Lymphocyte suspensions are most easily prepared from minced lymph follicles. The lymphatic tissue may come from the mesenteric lymph nodes (Stenstrom and King, 21), the thymus and spleen (Schrek, 16), the bone marrow (Osgood and Bracher, 11), or the submucosa of the rabbit vermiform process. The latter method was suggested by Professor Myers, and was the method used for this problem. Schrek (16) also used lymphocytic leukemic human blood as a tissue culture source. Markowitz (8, page 433) describes cannulation of the thoracic duct of dogs as a possible procedure. This should give cells most representative of those found in the peripheral blood.

D. Respiratory Metabolism.

Respiratory metabolism values for exudative rabbit leukocytes were shown by Ponder and MacLeod (12) to be independent of the cell concentration for counts in the range from 19,000 to 125,000 cells per cubic millimeter. The same investigators (14) found the Q_{O_2}(million cells) to average 0.40 microliters O_2 per million cells per hour. Expressed on a dry weight basis, this is about 5.5 microliters O_2 per milligram dry weight tissue per hour. The errors in estimating dry weights are normally

greater than the counting error, therefore the Q_{O_2}(million cells) form of expressing the result is preferable.

Materials and Methods

A. Tissue Culture

Exudative granulocytes were obtained for irradiation by a variation of the method of Mudd, Lucke, McCutcheon, and Strumia (10). Three hundred milliliters of sterile physiological saline were injected intraperitoneally in a rabbit, and the injection was repeated about sixteen hours later. For injection the rabbit was placed on its back, head down, on a rabbit board tipped about 30°. In this position the viscera were shifted forward out of the way, and an eighteen gauge needle was inserted about two inches below the umbilicus, near the midline. With the rabbit board returned to the horizontal a funnel on a ringstand was connected to the needle by flexible tubing and the saline solution introduced into the peritoneal cavity by gravity flow. Four hours after the second injection, approximately 150 milliliters of peritoneal fluid were withdrawn by syringe with an eighteen gauge needle one inch long which had four side holes drilled near the tip. The fluid exudate was received in 75 ml of citrate-saline solution (0.7% sodium chloride plus 1.1% sodium citrate), centrifuged at 1000 rpm for five minutes, and the supernatant fluid was discarded. The

white cells were washed in citrate-saline, and resuspended to a concentration of about 50,000 cells per cubic millimeter in buffered Ringer's solution composed as follows: 0.85% NaCl; 0.042% KCl; 0.024% $CaCl_2$; 0.1% glucose; 0.06% Na_2HPO_4; adjusted to pH 7.4 with H_3PO_4. The cells were counted with a hemocytometer. This technique was repeated at approximately weekly intervals for three months with no ill effect on any of the rabbits and without diminution in the number of cells recovered. Adult female rabbits of the New Zealand strain, weighing about four kilograms, were used.

The exudate was free of erythrocytes, and contained about 9,000 cells per cubic millimeter, which were predominately (95%) heterophilic polymorphonuclear granulocytes. Almost one and one-half billion granulocytes, or about two milliliters of packed cells were "harvested" each time. The granulocytes were phagocytic to Escherichia coli and exhibited ameboid movements. They remained viable for at least twenty-four hours if held at 4°C.

For the lymphocyte studies a dense suspension was obtained from the layer of lymph follicles in the wall of the vermiform process of the rabbit. The appendix was removed and the blind end cut off. The resulting flaccid tube was slit lengthwise, and when spread out and pinned, inside up, on a waxed board, was a rectangle of tissue which measured about three by ten centimeters. The topmost layer of epithelium was rolled off, exposing

the middle layer of lymphocytes, which was scraped off and received in citrate-saline.

This technique gave from one to two billion cells, which were handled, in general, the same as the granulocytes. The lymphocytes, however, coagulate much more readily than do the granulocytes, so that it was necessary to add heparin (about 0.05 mg/ml) to the citrate-saline and the buffered Ringer's solutions used for the lymphocytes.

B. Irradiation

A General Electric Maximar deep therapy installation was used to irradiate the cell suspensions. The constants were: 250 kvp, 15 ma, 22 cm target to suspension, minimum wave length 0.05 Angstroms. Dosage was calibrated with a Victoreen r-meter. With no filter (except the 0.5 mm Al equivalent self filter of the x-ray tube and the pyrex glass test tube), the dosage rate was 800 roentgens per minute, half value layer 0.635 mm Cu, equivalent wave length as determined with 1/4 mm Cu, 0.22 Å. With an added 1.0 mm aluminum filter the dosage rate was 690 roentgens per minute, HVL 0.642 mm Cu, equivalent wave length 0.21 Å.

The only convenient radio-isotope of phosphorus to use is P^{32}. It is a 1.71 Mev (maximum) beta emitter, with no gamma radiation. Available from Oak Ridge as approximately one-fiftieth molar phosphoric acid, it is

readily incorporated into a buffered Ringer's solution. The cells to be irradiated were suspended in this "hot" buffered Ringer's at dosage rates up to 24 rep per minute. Calculation of dose was made using the geometry factors of Richards and Rubin (15).

The active phosphorus metabolism of the irradiated cells was demonstrated by the radioactivity which remained, in spite of several washings, after the cells were separated from the "hot" buffered Ringer's solution. The self-irradiation resulting from this P^{32} uptake by the cells was negligible in comparison with the dose administered by the radioactive suspending medium.

C. Respirometry.

The peripheral white blood cells have several characteristics which are suitable as criteria of viability. Ameboid movement, chemotropic movement, and phagocytosis are probably less amenable to quantitative measurement than is cellular respiration. Accordingly, the Warburg constant-volume respirometer was used to measure oxygen uptake after irradiation of the cells.

The Warburg direct method described in Umbreit, Burris and Stauffer (22, page 12) was followed. This technique holds the gas and fluid volumes constant, absorbs all of the carbon dioxide evolved, and measures the decrease in pressure as oxygen is consumed. The

volume of oxygen utilized per hour, at standard conditions, is computed. The cells were added in 1.5 milliliters of a buffered Ringer's solution, in a concentration of about 50,000 cells per cubic millimeter. This corresponds to about fifteen milligrams dry weight of cells per flask, and gives about fifteen microliters oxygen uptake per hour at 37°C.

Cells receiving a particular dose were divided into from four to eight samples for the respiration measurements. The cells in suspension settle out under gravity, and tend to clump and form strings as the suspension ages. This makes division into aliquots difficult, and causes most of the variation in uptake among replicate flasks. Volumetric errors were minimized by using automatic pipettes. The mean for each set of samples was calculated, and the oxygen uptake expressed as a percentage of the uptake of the non-irradiated control. The t distribution was used to find the limits that would include the population mean in 95% of all samples from a normal population (Winsor, 24, page 114), and this 95% confidence interval was plotted in percentage of control.

Experimental Results

A. Granulocytes

Figures 1 through 7, following, contain the results of this portion of the investigation. The "standard curve", figure 1, is for exudative granulocyte suspensions buffered at pH 7.4, exposed to unfiltered x-rays at room temperature, 800 roentgens per minute. These data show an increased respiration up to about 6000 roentgens, then a decrease with a dose of 22,800 r causing 50% reduction in oxygen consumption. An exponential survival curve, fitted by the method of least squares without regard to the confidence limits of the points, is shown for comparison.

Variation of effect due to adding a 1.0 mm aluminum filter and so removing the softer x-rays is shown in the next plot. Although the quality and dose rate change is small, the radiation effect on oxygen uptake is much less than it is for the "standard curve".

Figure 3 shows that one roentgen-equivalent-physical of beta radiation from phosphorus corresponds to 0.56 roentgen of 250 kvP, 15 ma unfiltered x-radiation for the 50% dose for granulocytes. This corresponds to 142 ergs per gram tissue or a value of 55.8 electron volts per ion pair in tissue. However, the dose rate was only 24 rep per minute, and the P^{32} suspension was held at 4°C. The variation of these parameters may be part of the cause of this non-equivalence of rep and roentgen for this biological system.

One granulocyte culture was obtained from a rabbit which had received, one month previously, 1000 r of whole-body x-radiation. The cells were irradiated in vitro and respiration determined in the usual manner. On the basis of these limited data (figure 4), the in vivo exposure resulted in granulocytes which were more radio-resistant than those obtained from rabbits that had received no previous exposure.

The next three curves show the results of changing the conditions during exposure. Irradiation in the cold (figure 5) gave results similar to the standard curve below 15,000 r. Respiration was less depressed at this dose level, and was slightly stimulated in the three samples receiving higher doses.

Figure 6 is a plot of oxygen uptake after 9,000 r of filtered x-radiation against the pH of the buffered Ringer's cell suspension. The respiration is expressed in per cent of the uptake of the non-irradiated control held at the same pH, thereby eliminating the effect of pH on the method of respiration measurement. A strong dependence on hydrogen ion concentration is evident, with a maximum cell mortality when suspended in a slightly acid medium. A pH drop of 0.3 from the physiological 7.4 doubled the radiation effect on granulocytes. This pH change also corresponds to doubling the number of hydrogen ions in the suspension. Some of the scatter of the

points of the standard curve, figure 1, is probably due to undetected variation in pH.

The protection phenomena accompanying an increase in cell concentration is evident in figure 7. Eleven determinations of oxygen uptake at each of two dose levels were plotted against the concentration of the cell suspensions. A linear regression line was fitted by least squares, and the correlation coefficient (Winsor, 24, page 122) and mean value of oxygen uptake computed for each plot. At 7,200 r, the mean respiration was 90% of control, the correlation coefficient 0.57. In the range of concentrations investigated, from one to sixteen times the leukocyte concentration in the peripheral blood, a change of 30 million cells per ml. caused a change of ten per cent in radiation effect. At twice this dosage, 14,400 r, mean respiration was 79%, correlation coefficient 0.37, and a ten per cent change resulted from a shift of 27 million cells per ml. This dependence of radiation effect on cell concentration is one cause of the scatter in the data summarized in figure 1, because the cell concentration was not the same for different suspensions.

The interval between beginning of irradiation and beginning of the respirometer readings averaged 2 1/4 hours, and ranged from 1 1/2 to 3 3/4 hours. An analysis of the data showed no correlation between radiation effects and this "incubation time." Nor was there any variation of effect of radiation on cultures prepared from different rabbits.

Radiation Effect on Granulocytes

Unfiltered x-ray; HVL 0.635mm Cu

pH 7.4; 25°C

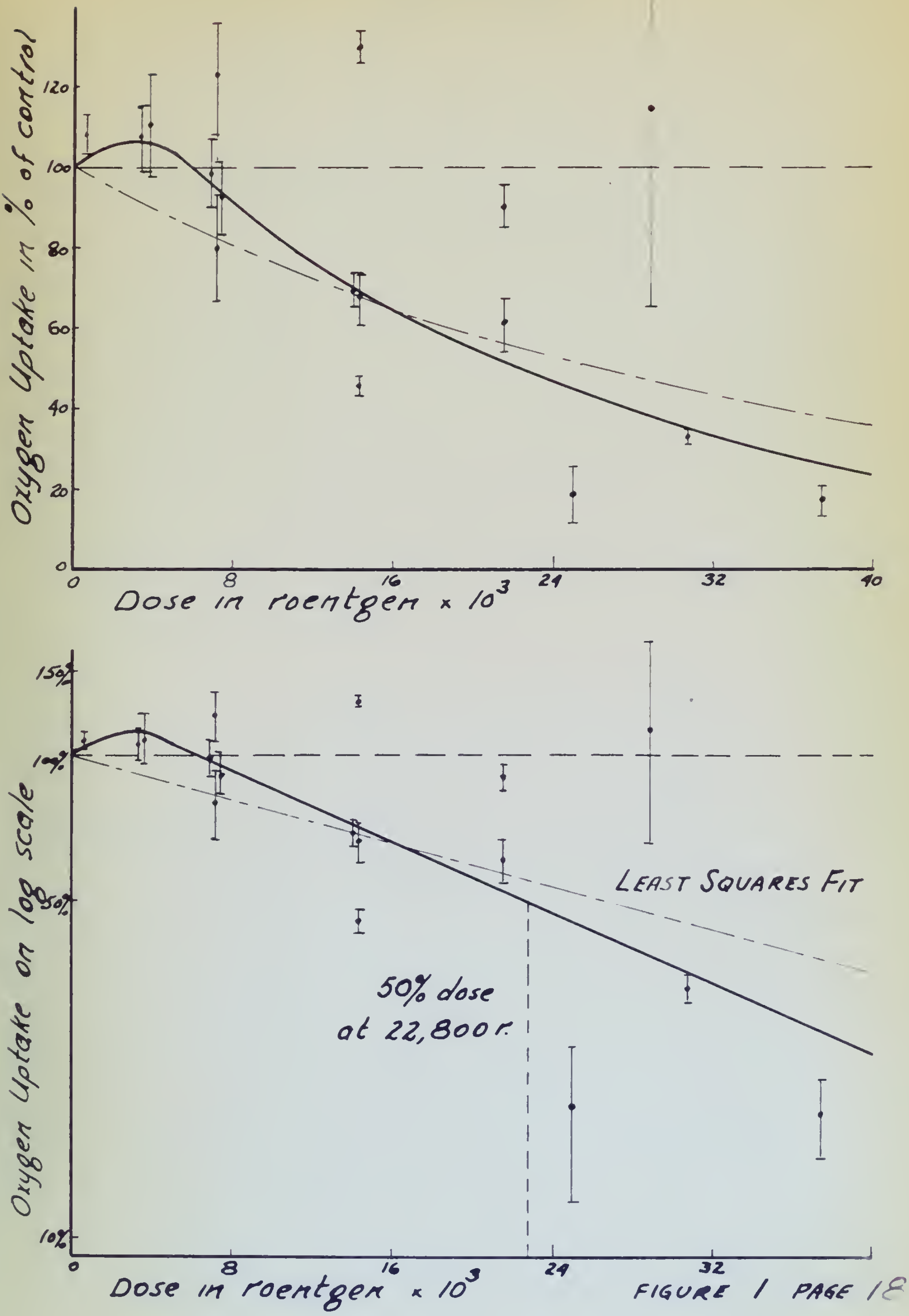

FIGURE 1

Radiation Effect on Granulocytes

Aluminum Filter

Filtered x-ray; H.V.L. 0.642 mm Cu.

pH 7.4; 25°C

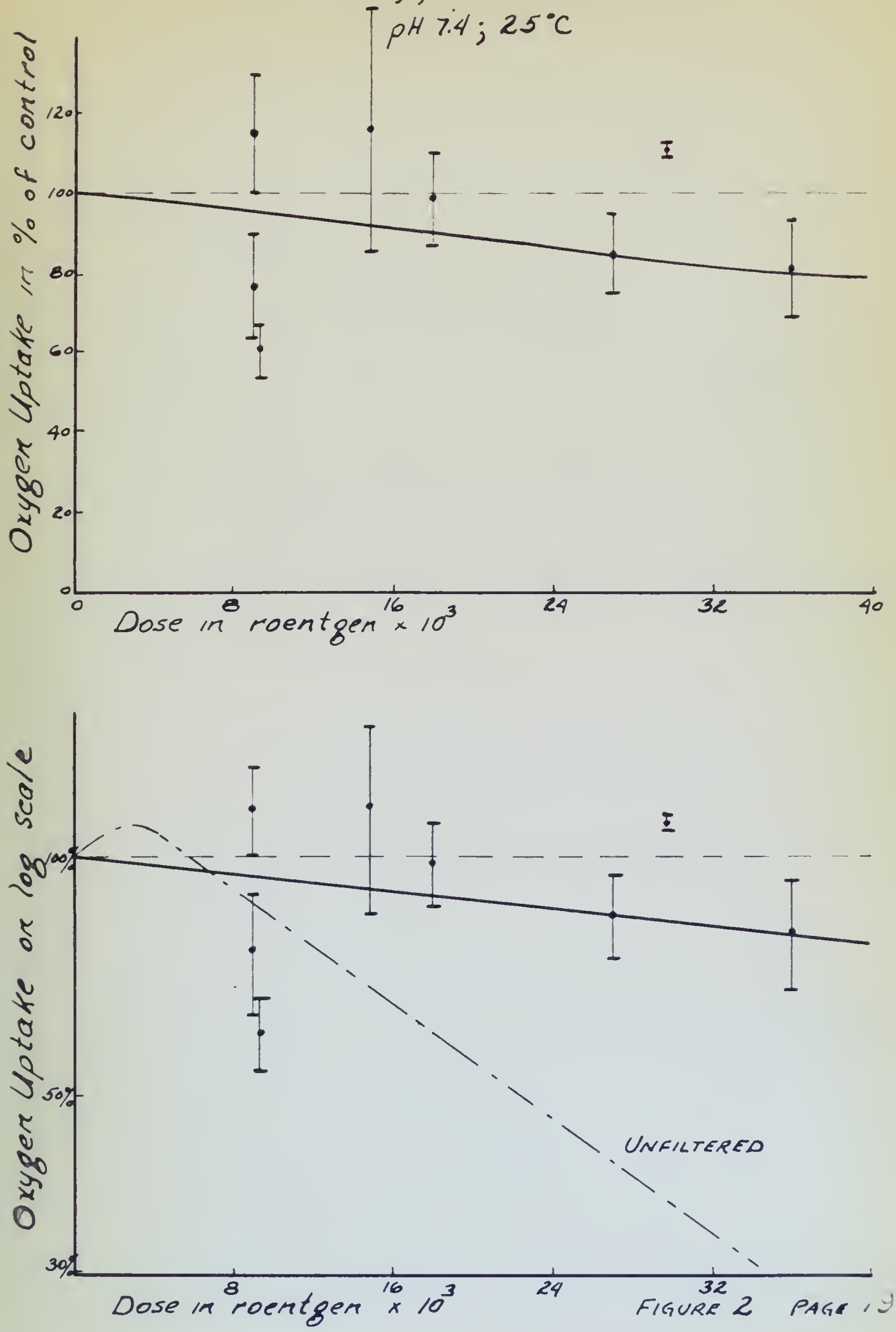

FIGURE 2

Radiation Effect on Granulocytes
Phosphorus Irradiation
Cells suspended in $P^{32}O_4$ buffered
Ringer's solution.
pH 7.4 ; 4°C

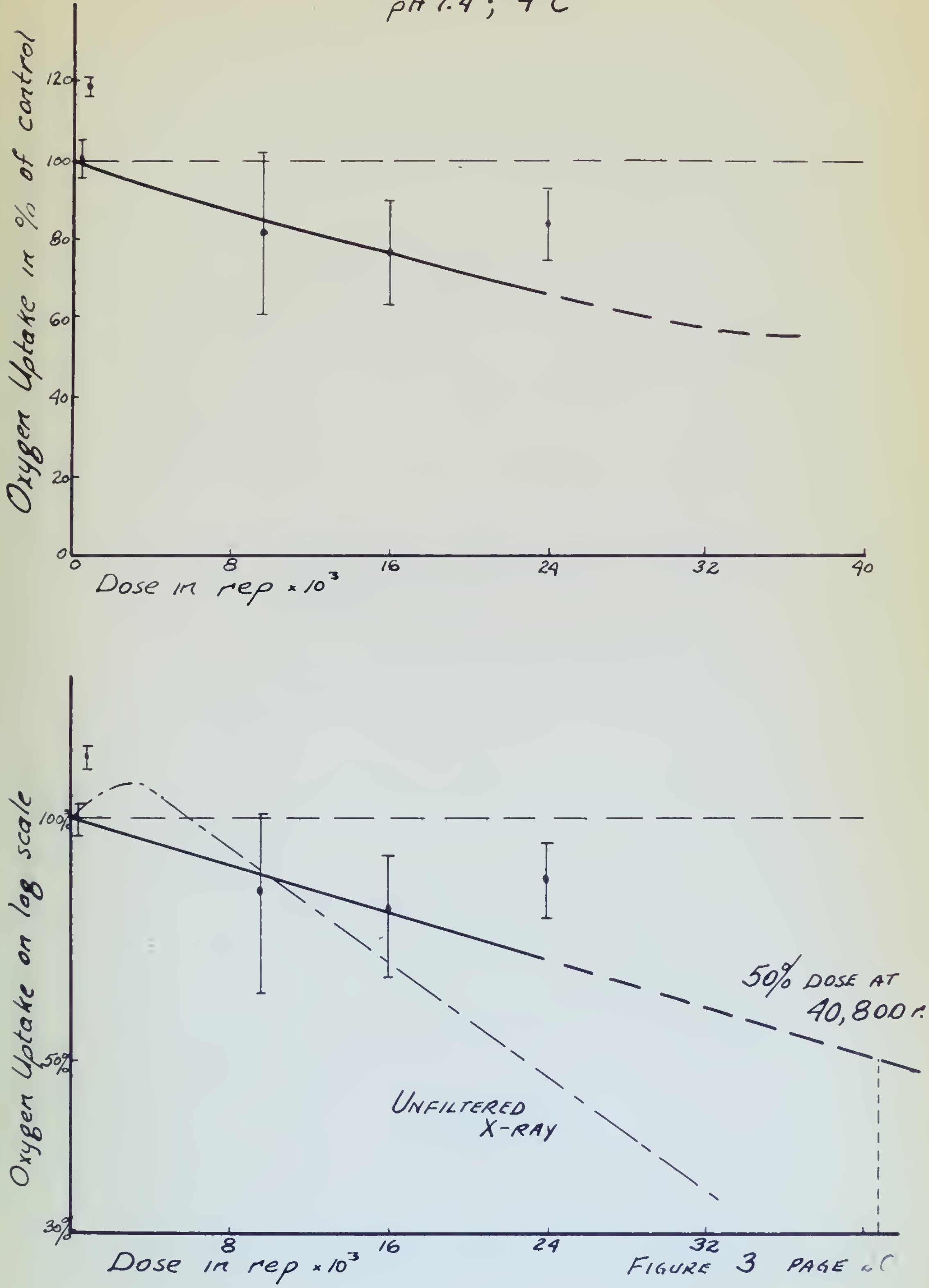

FIGURE 3 PAGE [illegible]

Radiation Effect on Granulocytes
One month after 1000 r. in vivo Irradiation
Unfiltered x-ray; HVL 0.635 mm Cu
pH 7.4; 25°C

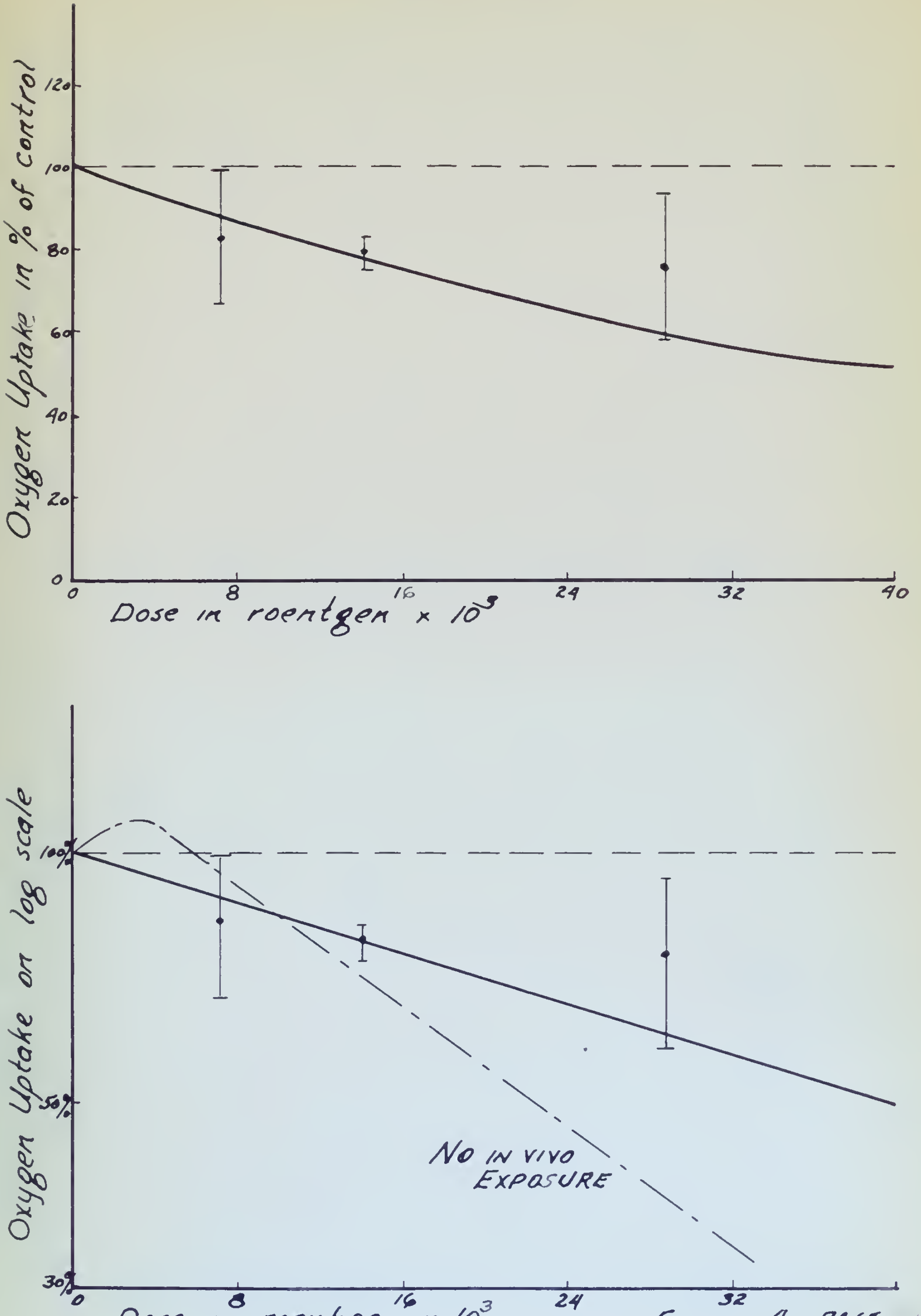

FIGURE 4

Radiation Effect on Granulocytes
Low Temperature

Unfiltered x-ray; HVL 0.635 mm Cu
pH 7.4; 10°-20°C

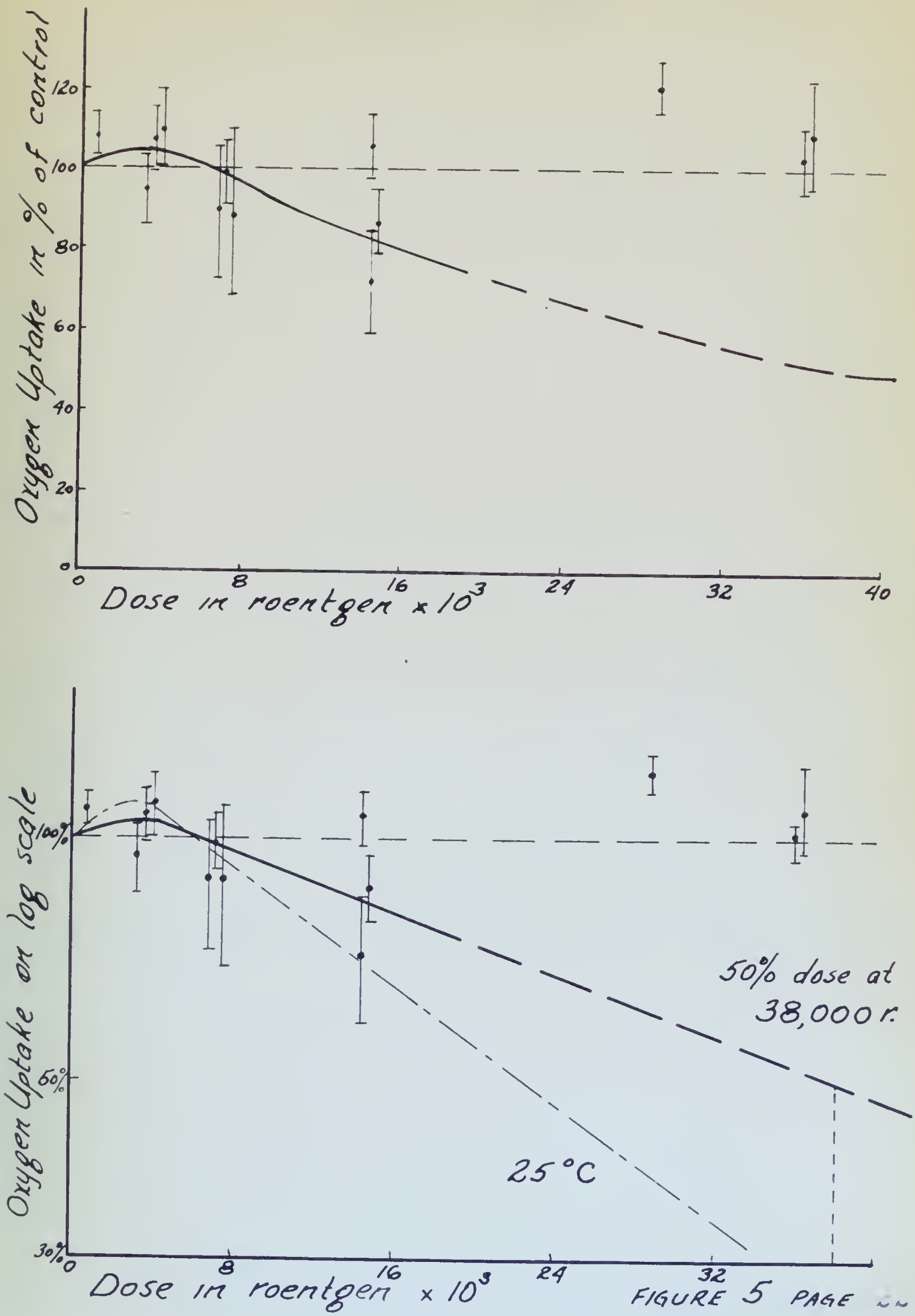

FIGURE 5 PAGE

Radiation Effect on Granulocytes
Variation with pH
Al filtered x-ray; HVL 0.642 mm Cu
9000.r. ; 25°C

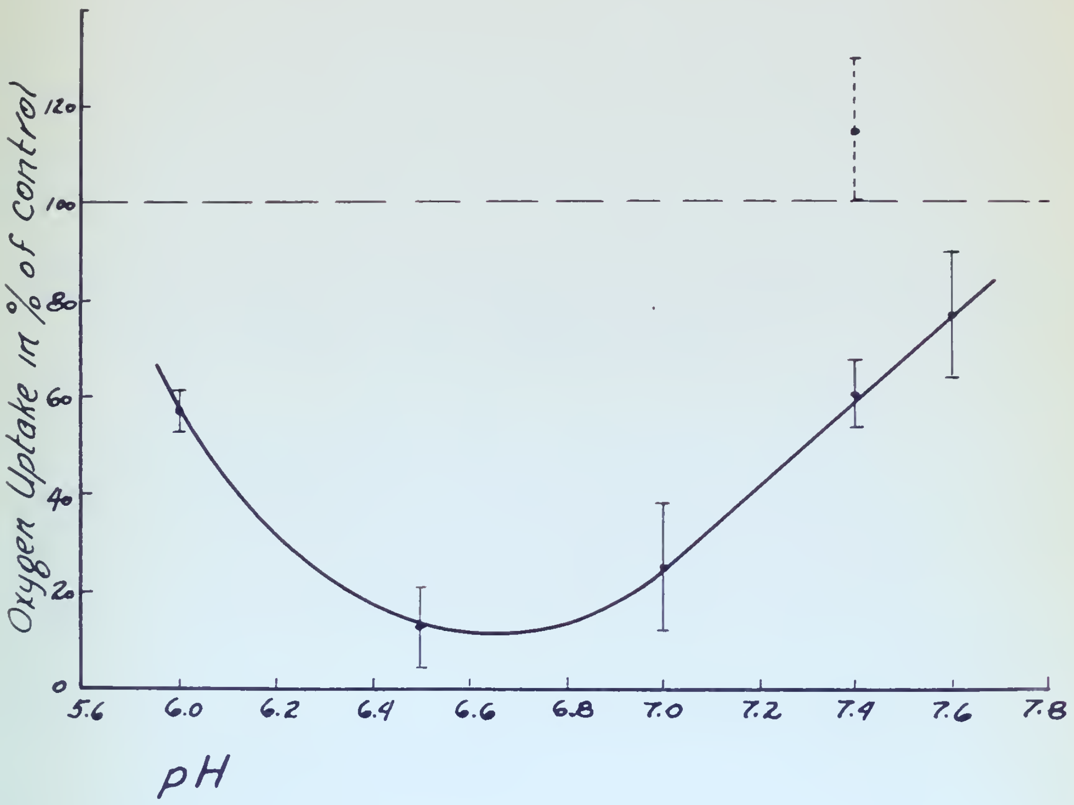

FIGURE 6 PAGE

Radiation Effect on Granulocytes
Variation with concentration
of cells suspended in Ringer's
buffered at pH 7.4

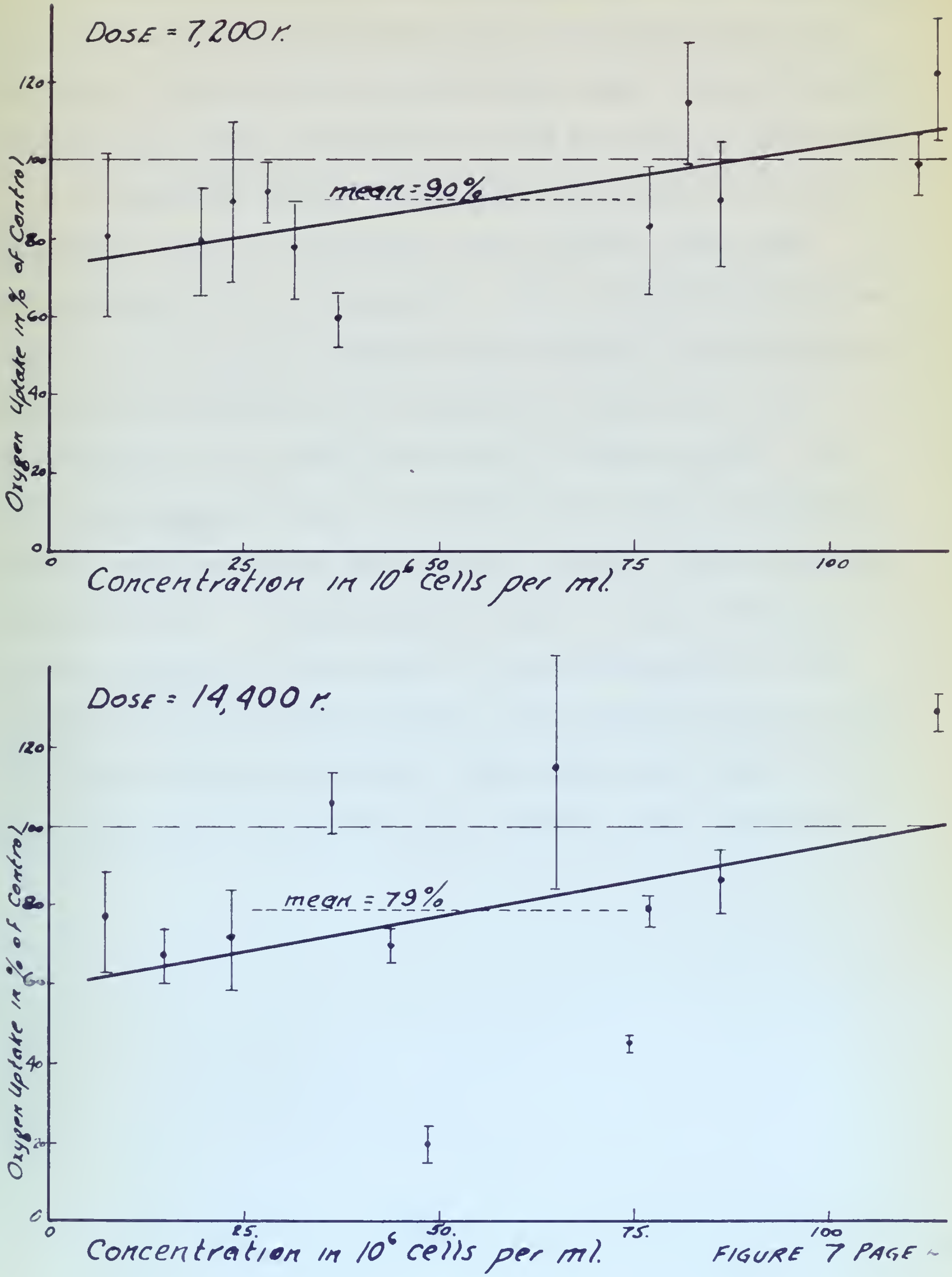

FIGURE 7 PAGE ~ 1

B. Lymphocytes

Figures 8 and 9 depict the results from irradiation of lymphocytes obtained from the appendix. The "lymphocyte standard curve" of figure 8 shows an immediate drop in activity, followed by an exponential decay. The 50% dose is 6,000 r, about one-fourth of that for the granulocytes.

A shallower initial depression followed by an exponential drop in respiration was obtained from beta irradiation of the lymphocytes. One roentgen-equivalent-physical of 1.71 Mev maximum beta radiation corresponds to one-fourth roentgen of the unfiltered 250 kvP, 15 ma x-radiation at the 50% dose level for these cells. As with the granulocytes, the lowered dose rate and ambient temperature may cause the reduced effect of beta compared to x-radiation. Another major cause of this deviation is the clumping of the cells in the suspension as they settle out under gravity during the overnight exposure to the "hot" buffered Ringer's. This makes the actual dose less than that calculated for a uniform cell suspension.

Radiation Effect on Lymphocytes
Unfiltered x-ray; HVL 0.635 mm Cu
pH 7.4; 25°C

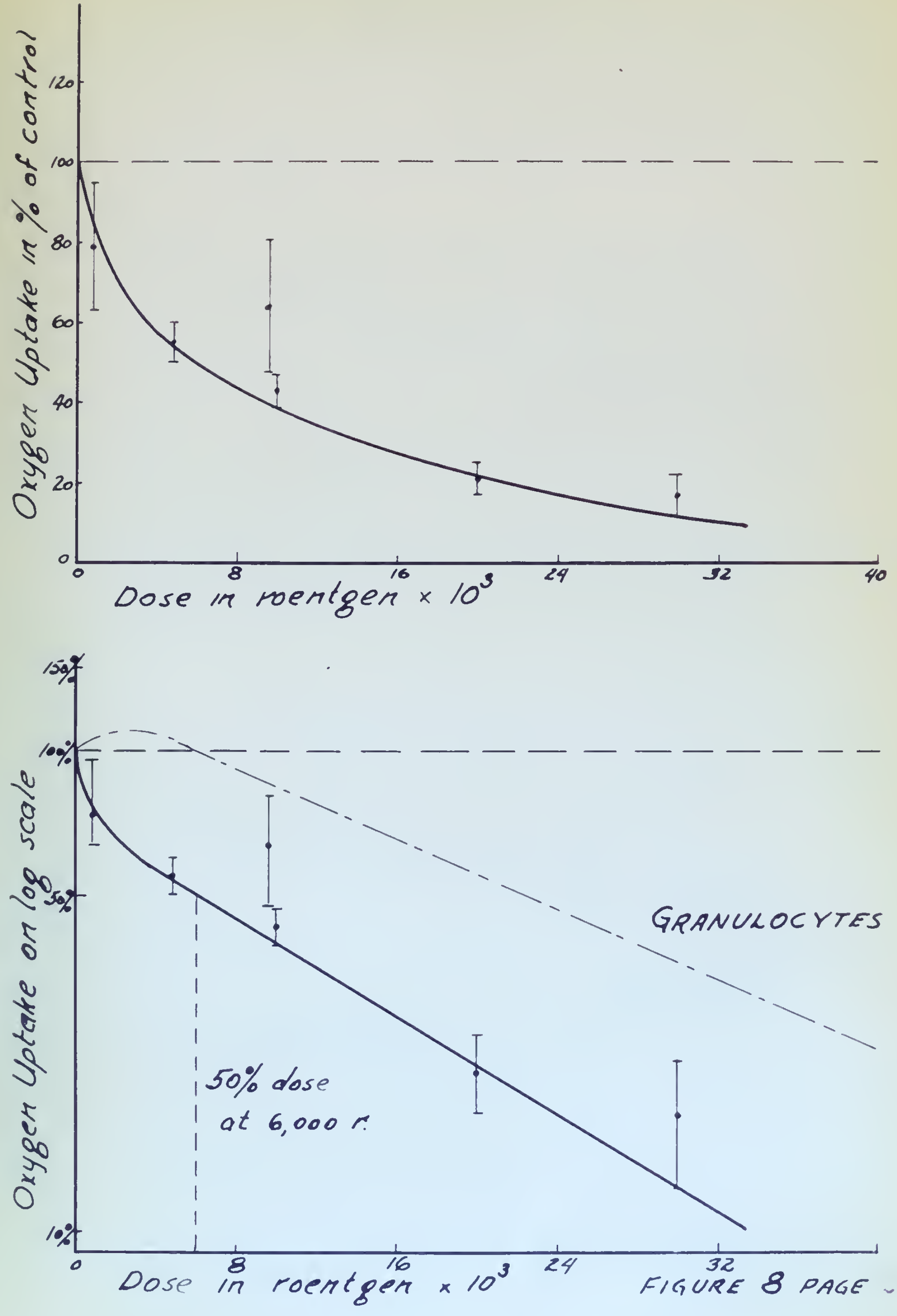

FIGURE 8 PAGE [illegible]

Radiation Effect on Lymphocytes
Phosphorus Irradiation
Cells suspended in $P^{32}O_4$ buffered
Ringer's solution
pH 7.4 ; 4°C

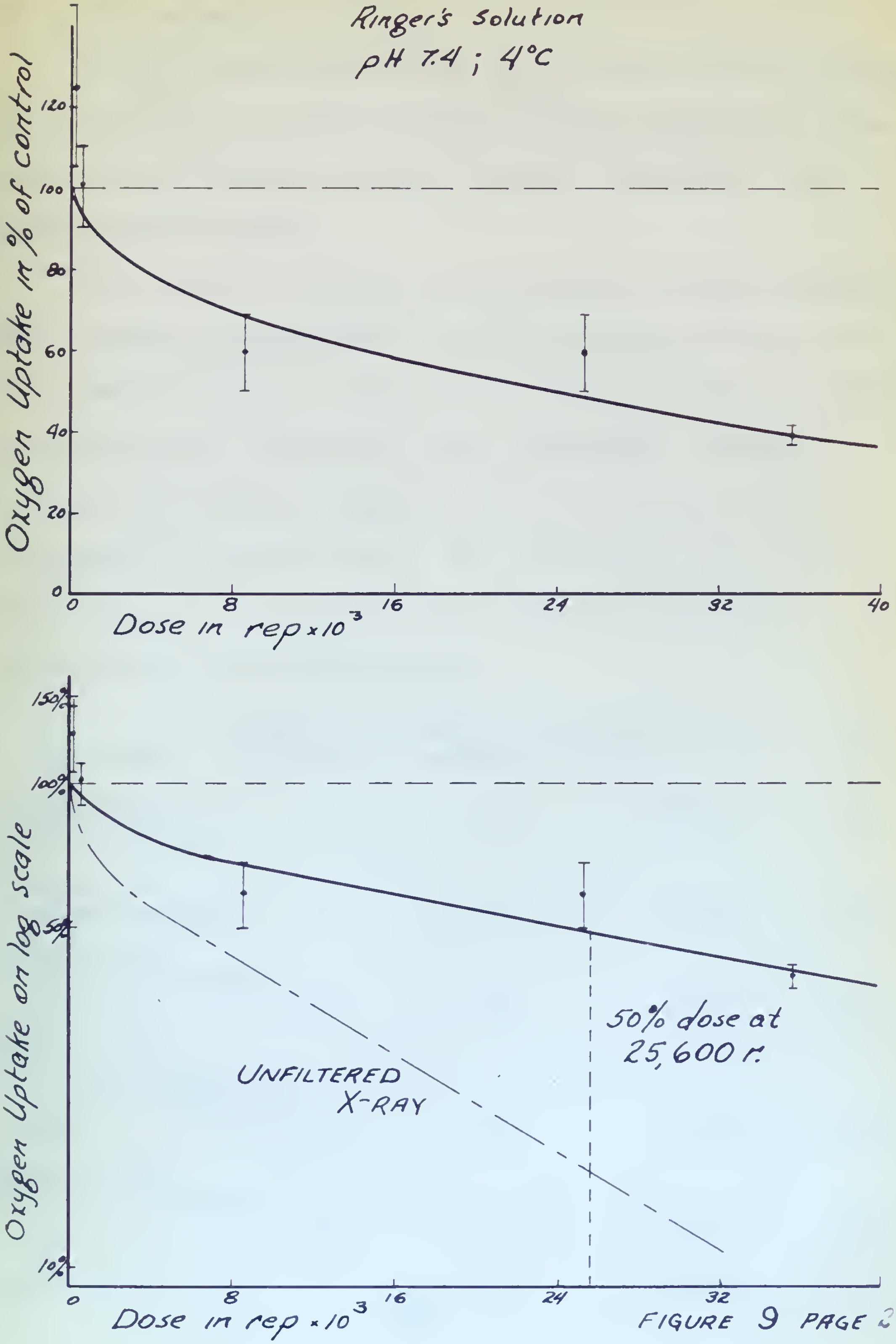

FIGURE 9

6. Respiratory metabolism values

The absolute values of oxygen uptake are averaged in the table below.

The Q_{O_2} was computed from Q_{O_2}(million cells) by using the experimental values of 10.3 million granulocytes per milligram dry weight and 26.2 million lymphocytes per milligram dry weight.

Note that no increase in Q_{O_2} such as would indicate more immature granulocytes was found between first and subsequent cultures, except that the Q_{O_2} for the exudates obtained after a three-week rest following a culture series were about 30% higher than the average of all granulocyte determinations. No correlation was found between Q_{O_2} and the age of the cell suspension when respiration measurements began.

Cell type	Number of cultures	Number of samples	Q_{O_2}(10^6 cells)	Q_{O_2}
Granulocyte, first culture	4	16	0.261	2.69
Granulocyte, second culture	4	24	.254	2.62
Granulocyte, series at weekly intervals	13	76	.249	2.56
Granulocyte, 3 weeks after previous "harvesting"	3	14	.333	3.43
Granulocyte, average of all runs	24	130	0.262	2.70
Lymphocyte	4	16	0.662	17.3

Discussion

In these studies large doses of penetrating radiations were found necessary to depress the respiration of the mature white blood cells. So, although it is a quantitative measure, oxygen uptake is not a sensitive criterion of cell damage. The phagocytic index or the nucleic acid metabolism demonstrated with radioactive tracer techniques could be equally quantitative, and might be more sensitive indicators of radiation effect on leukocytes.

The threshold of radiation effect observed in the oxygen uptake of granulocytes and the initial depression in the uptake of the irradiated lymphocytes cause most of the difference between the response of the two cell types. The exponential portions of the two curves have nearly the same slopes. Radiation has four times the effect on lymphocytes as on granulocytes in terms of the dose required to reduce the oxygen uptake to 50% of control. But if the threshold effects are ignored, and the exponential portions of the two curves are compared, radiation has only 1.4 times the effect on lymphocytes that it has on granulocytes.

The radiation effects *in vitro* were shown to vary inversely with the granulocyte concentration of the suspension over a range of one to sixteen times their concentration in the peripheral blood. During the *in vivo* exposure the circulating leukocytes are surrounded by 600 times their

number of erythrocytes. It seems reasonable to surmise that the erythrocytes afford the leukocytes this same protection during the *in vivo* irradiation.

Because such large doses of radiation were required for destruction of the peripheral white blood cells *in vitro*, damage to their precursors in the myeloid and lymphoid hematopoietic tissue rather than direct effect on the mature circulating cells may account for the lymphopenia as well as for the granulocytopenia which follows whole-body exposure to large amounts of penetrating ionizing radiation.

Large dosages to three cell suspensions at low temperatures (10-20°C) gave no depression of respiration. This may be due to an "indirect action" or it may be an anomalous effect, because these samples had high cell concentrations. Further work within narrow concentration limits, over a range of temperatures from 0-40°C, and at constant dosage, is necessary before conclusions can be fairly drawn concerning the temperature effects. Such a study with these leukocytes might demonstrate a definite temperature dependence for radiation effects.

The independence found between radiation effect and "incubation time" argues against the "indirect action" concept which predicts that longer exposure to the irradiated medium causes greater depression of cell activity. This is not a conclusive result, however, because the time lag between radiation exposure and

respiration measurement in this experiment was small and constant in comparison to the life of the granulocytes, and the cells were held at room temperature instead of at 37°C.

The linear dependence of radiation effect on the hydrogen ion concentration of the suspension when near the physiological pH of 7.4 strongly suggests an "indirect action" phenomenon. However, the radiation effects, when plotted, followed, in general, a holistic, single-event action curve, as would be predicted by the "hit theory". Probably both explanations of the mechanisms of radiation damage to the cell are necessary. They can be combined qualitatively in some such statement as: The primary ionizations in the large protein molecules of the cell produce cell decomposition products, and in the water environment produce active radicals. These are, or cause, chemical changes, and so an alteration of the cell function results.

Conclusions

These in vitro studies confirm the previous findings that:

1. Leukocyte respiration is depressed by large doses of radiation.

2. Lymphocytes obtained from the appendix are much more sensitive to radiation than are the granulocytes obtained from the peritoneal exudate obtained experimentally, only one-quarter of the dose being required for the same effect.

It has also been shown by these studies that:

3. Where granulocytes are used as a biological indicator, 1.7 rep of P^{32} beta radiation is equivalent to one roentgen of unfiltered x-radiation (HVL 0.635 mm. Cu).

4. The radiation effects vary inversely with granulocyte concentration in the suspension, over the range from one to sixteen times their concentration in the peripheral blood.

5. Radiation effects on the oxygen uptake are strongly dependent upon the pH of the suspending medium, and directly proportional to the hydrogen ion concentration over a range of one pH unit centered around the pH of blood.

6. Irradiation at 10-20°C has no definite effect on respiration, nor does the "incubation time" after irradiation.

It is concluded that:

7. Destruction of the peripheral white blood cells is possibly a minor factor in radiation injury.

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